1348-Pos Board B78
Determining the Structural Topology of KCNE1 in a Lipid Bilayer using Electron Paramagentic Resonance (EPR) Spectroscopy
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KCNE1 (also known as MinK) is a membrane-bound protein with a single transmembrane section consisting of 129 amino acids with a molecular weight of 15.7 kD. The main function of KCNE1 is to modulate the activity of the KCNQ1 voltage-gated potassium ion channel in the human heart. This potassium modulation is critical for repolarization in the heart, specifically in the left ventricle. Mutations in the KCNE1 gene can result in genetic disorders such as long QT syndrome, sudden infant death syndrome (SIDS), as well as deafness. However, most current KCNE1 studies are from a functional standpoint, which leaves the structural and dynamic information of the protein unknown. EPR spectroscopy is a very powerful structural biology tool that can be used to determine the structural topology of membrane proteins with respect to its membrane environment. In this study, we use the EPR power saturation and EPR membrane alignment techniques coupled with site-directed spin-labeling (SDSL) to study the structural topology of KCNE1 with respect to the lipid bilayer.

1349-Pos Board B79
Structure of a Three Helix Membrane Protein from Oriented Sample and Magic Angle Spinning NMR Data
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Of the many membrane protein structures deposited in the Protein Data Bank, only a few are of small helical proteins. Unfortunately, the typical transmembrane helix content of membrane proteins in the genomes of pathogenic bacteria is largely biased towards those with four or fewer helices. Many of these proteins are known or potential drug targets. The common structural techniques of x-ray crystallography and solution state NMR have had limited success in the determination of these structures [1]. Solid state NMR has emerged as a powerful tool capable of characterizing membrane protein structure in lipid bilayers. Oriented sample solid state NMR provides a set of peptide plane orientations. Magic angle spinning solid state NMR provides a set of interhelical distance restraints. Here, we show that together these restraints are capable of determining a backbone structure for the transmembrane domain of a three helix membrane protein. Furthermore, the structure orientation in the lipid bilayer is uniquely determined when using oriented sample restraints. We applied these methods to the Rv1861 protein from Mycobacterium tuberculosis, the causative agent of tuberculosis which is responsible for over one million deaths annually. Altogether, these experiments show that high resolution structures can be routinely determined for three to four helix membrane proteins and will help fill the void in the Protein Data Bank for these important drug targets.
[1] Cross, T.A., Murray, D.T. and Watts, A. Eur. Biophys. J. In Press (2013) DOI 10.1007/s00249-013-0925-x.

1350-Pos Board B80
Structural Studies of Heteromeric Connexin26/30 Hemichannels via Atomic Force Microscopy Imaging
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Connexins (Cx) form hexameric hemichannels that mediate release of small molecules, including ATP and glutamate, to mediate paracrine communication and signaling. There are ~20 vertebrate connexins, and most cells seems to express more than one isoform. Biochemical and functional data demonstrate that hemichannels can be heteromeric - composed of more than one connexin isoform and that the gating and permeability properties are modulated by the heteromericity. However, the subunit stoichiometry and arrangement within heteromeric hemichannels is essentially unexplored. Heteromeric Cx26/30 channels were immunopurified from HeLa cells expressing a haemagglutinin tagged Cx26 (Cx26-HA) and Cx30. Presence of the heteromeric Cx26/30 hemichannels was confirmed by mass spectrometry analysis. Purified samples were imaged by air tapping mode atomic force microscopy (AFM). Molecular volumes of the Cx26/30 hemichannel complexes showed a particle population centered at 337 ± 10 nm3, which is within the volume range expected for heteromeric Cx hexamers based on their molecular weights. Ongoing studies will visualize Cx26/30 hemichannels decorated with isoform-specific anti-haemagglutinin antibodies by AFM to determine both the number of each subunit in the complex and its subunit arrangement. Funded by Millennium Science Initiative P10-035F/Fondecyt 1120169/Anillo ACT 1108 grants.

1351-Pos Board B81
Comparative Analysis of Full-Length Cytochromes P450 in Complexes with Cytochrome B5 in Membrane
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Molecular models of complexes of full-length cytochrome P450s with cytochrome B5 (CYB5) were developed based on experimental structures of CYB5 (2m33) and several P450s in closed (2q6n, 1tqn, 3e4e, 3eqm, 3ruk) and open (2bdm) conformations and structural constraints derived from mutagenesis, mass-spectrometry, and NMR studies. Models of transmembrane parts of the complexes formed by two antiparallel α-helices were generated using original energy functions for quantification of helix interactions in membranes. Our PPM method was used to assess the spatial orientations of complexes in membrane. We found that CYB5 (helices 41-43, 48-54, and 60-66) binds to the proximal groove of P450s formed by helices B, C, J', K, L, the C-D loop, and the K''-L meander region. In all models, acidic residues E61 and D65 of CYB5 form ionic pairs with basic residues of P450 (from helices B, C, and the K''-L meander), whereas propionate groups of the heme interact with conserved arginine from helix C (R133 in CYPB4). However, CYB5 orientations vary in complexes with different P450s and depend on the conformational state of P450s. In particular, CYB5 interacts similarly with closed forms of CYP2E1, CYP17A1, and CYP2C19: E58 of CYB5 forms a contact with helix B of P450 and E42/E48 of CYB5 interact with helices J', K, and L of P450. A partly different binding mode was identified for complexes of CYP3A4 and CYP2B4, where E42/E48 of CYB5 contact with the K''-L meander and T70/D71 of CYB5 interact with the C-D loop. Comparison of complexes with CYP2B4 in closed and open conformations shows that open conformation has larger protein-protein contact surface, shorter heme-to-heme distance, deeper penetration of the complex into membrane, and provides additional interprotein hydrophobic interactions (V50-I435 and V66-L437) which stabilize the complex.

1352-Pos Board B82
Theoretical Investigation of TrHbN Association to Biological Membranes
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TrHbN is a group I truncated hemoglobin from Mycobacterium tuberculosis (Mtb). TrHbN protects Mtb aerobic respiration from •NO inhibition and is thought to play pivotal roles in the persistence of mycobacterial infection. Its 3D structure is characterized by the presence of hydrophobic tunnels. Previous MD simulations revealed that three of theses tunnels are used by •NO to reach the active site. Despite the fact that trHbN is soluble and highly active in aqueous solvent, there is compelling evidence suggesting that trHbN is associated with biological membranes. We have investigated the association of trHbN with membranes differing in lipid compositions (DOPC, CL and CL:DOPE) using all-atom MD simulations.
We found that trHbN penetrates the peripheral regions of the lipid bilayer for all the lipid compositions. Two portions of trHbN make a dominant contribution to the membrane binding: the N-terminal pre-A helix, and the G and H helices from the core of the protein. The pre-A helix has four charged residues that interact with the lipid phosphates, and several hydrophobic residues that insert under the lipid headgroups. The G and H helices have several hydrophobic residues in contact with the aliphatic chains. These residues were identified to form a large hydrophobic funnel-shape entrance of the Short tunnel (ST), previously shown to be preferentially used by •NO to reach the distal heme pocket. As a consequence, the ST has its entrance pointing towards the lipid bilayer, while the Long tunnel (LT) entrance is pointing towards the aqueous solvent. The orientation of trHbN differs slightly in presence of cardiolipins due to additional lipid phosphate-protein core interactions. These results suggest that the substrates reach the active site from the membrane interior using the ST and the products escape to the solvent using the LT.

1353-Pos Board B83
Structure, Dynamics, and Receptor Binding of Opa Proteins
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Opacity associated (Opa) outer membrane proteins are eight-stranded β-barrels from the bacteria *Neisseriae* that induce engulfment of the bacterium in non-phagocytic host cells by binding to host receptors. To determine high-resolution structures of Opa proteins and Opa-receptor complexes, the selection of the membrane mimic is important in capturing the complex. Mimic selection is largely empirical, and only a select few detergents and nanodiscs have led to